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Interleukin-8 receptor A: SeqID: ENSP00000295683

Sequence and pentamer URS distribution

MSNITDPQMWDFDDLNFTGMPPADEDYSPCMLETETLNKYVVIIAYALVFLLSLLGNSLVMLVILYSR

VGRSVTDVYLLNLALADLLFALTLPIWAASKVNGWIFGTFLCKVVSLLKEVNFYSGILLLACISVDRY

LAIVHATRTLTQKRHLVKFVCLGCWGLSMNLSLPFFLFRQAYHPNNSSPVCYEVLGNDTAKWRMVLRI

LPHTFGFIVPLFVMLFCYGFTLRTLFKAHMGQKHRAMRVIFAVVLIFLLCWLPYNLVLLADTLMRTQV

IQESCERRNNIGRALDATELLGFLHSCLNPIIYAFIGQNFRHGFLKILAMHGLVSKEFLARHRVTSYT

SSSVNVSSNL

ENSP00000295683 list of pentamer URS peptides (total number 35) AHMGQ AKWRM AYHPN CERRN CLGCW CYGFT
DLNFT DPQMW DTAKW FCYGF FKAHM GQNFR HTFGF IWAAS KWRMV KYVVI MGQKH MPPAD NFTGM NGWIF PQMWD
PYNLV QKHRA QMWDF QNFRH TAKWR TDPQM VCLGC VCYEV VMLFC VNGWI WDFDD WIFGT WRMVL YSPCM

URS Penta-peptide

Figure 1

Histamine H1 receptor: SeqID: ENSP00000273023

Sequence and pentamer URS distribution

MSLPNSSCLLEDK MCEGNK TTMASPQLMPLVVVLSTICLVTVGLNLLVLYAVR SER K LHTVGN

LYIVSLSVADLIVGAVVMPMNILYLLMSK WSLGR PLCLEWLSMDVVASTASIFSVFLLCIDR YR

SVQQPLR YLK YR TK TR ASATILGAWFLSFLWVIPILGWNHFMQQTSVR R EDK CETDFYDV

TWFK VMTAIINFYLPTLLMLWFYAK IYK AVR QHCQHR ELINR SLPSFSEIK LR PENPK G

DAK K PGK ESPWEVLK R K PK DAGGGSVLK SPSQTPK EMK SPVVFSQEDDR EVDK LYC

FPLDIVHMQAAAEGSSR DYVAVNR SHGQLK TDEQGLNTHGASEISEDQMLGDSQSFSR TDSDTT

TETAPGK GK LR SGSNTGLDYIK FTWK R LR SHSR QYVSGLHMNR ER K AAK QLGFIMA

AFILCWIPYFLFFMVIAFCK NCCNEHLHMFTIWLGYINSTLNPLIYPLCNENFK K TFK R ILHI

R S

URS Penta-peptide: AINF CEGNK CNENF GWIPY DFYDV DQMLG FLCIF LWIFMQQT FWLSM GWNHF IHLHME HMFTI HTVGN IFEMV
ILGWI LLMSK LWFYA MCEGN MDYVA MFTIW MLWFY NCCNE NHFMQ PLCLF SPWEV VIAFC WFYAK VNHFM WVIPI YCFPL

K trypsin cutting site; URS Penta-peptide;

Figure 2

Figure 3

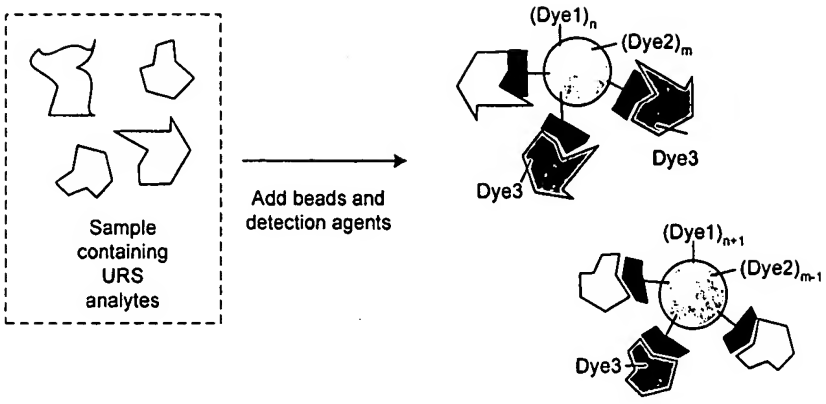

Sample containing URS analytes
Add beads and detection agents
(Dye1)n
(Dye2)m
Dye3
Dye3
(Dye1)n+1
(Dye2)m-1
Dye3

Figure 4

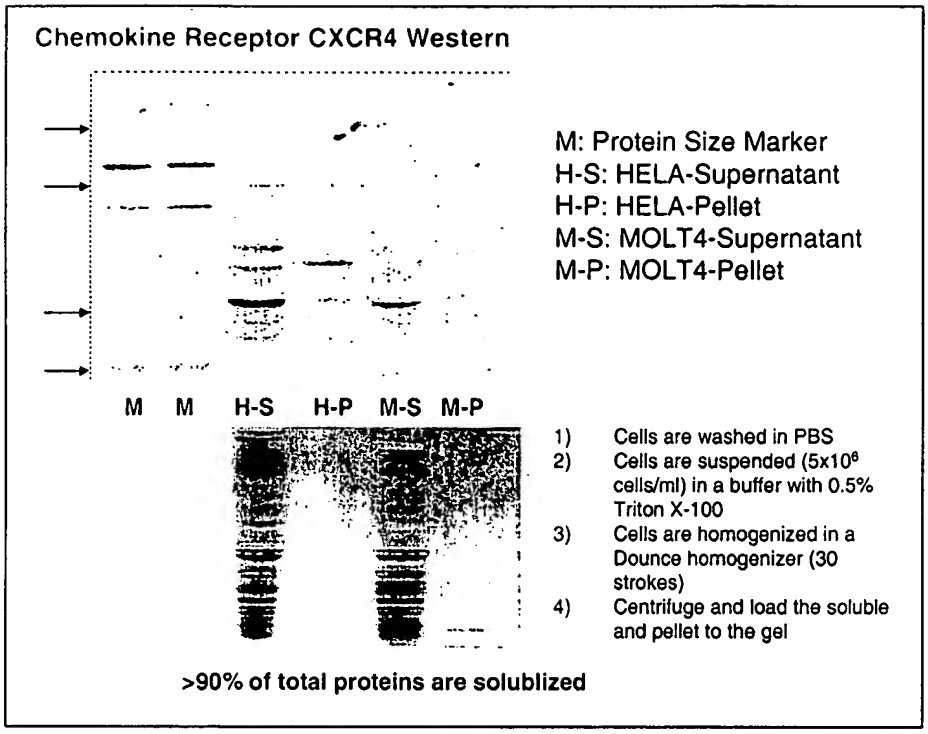

Chemokine Receptor CXCR4 Western
M: Protein Size Marker
H-S: HELA-Supernatant
H-P: HELA-Pellet
M-S: MOLT4-Supernatant
M-P: MOLT4-Pellet
M M H-S H-P M-S M-P
1) Cells are washed in PBS
2) Cells are suspended (5x10⁶ cells/ml) in a buffer with 0.5% Triton X-100
3) Cells are homogenized in a Dounce homogenizer (30 strokes)
4) Centrifuge and load the soluble and pellet to the gel
>90% of total proteins are solublized

Figure 5

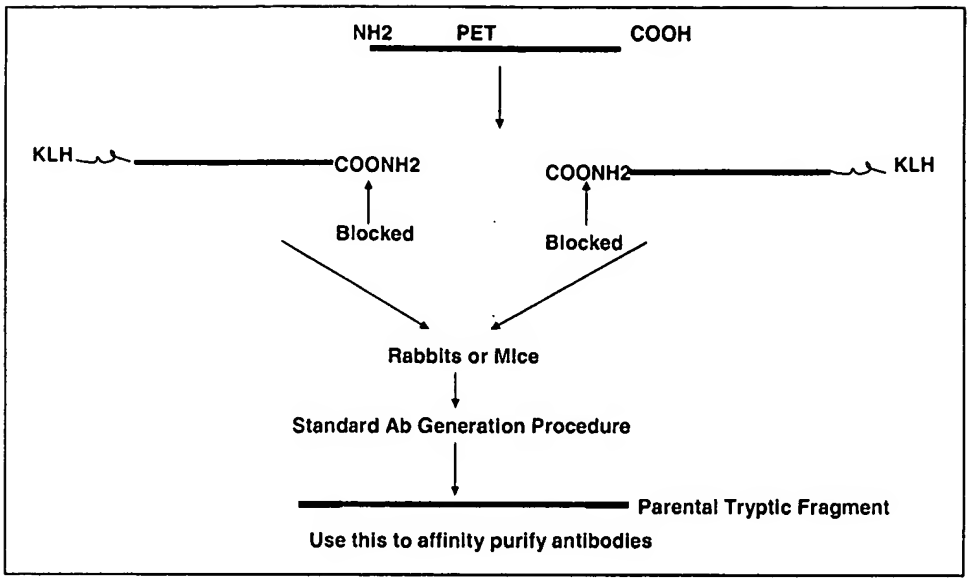

NH2
PET
COOH
KLH
COONH2
COONH2
KLH
Blocked
Blocked
Rabbits or Mice
Standard Ab Generation Procedure
Parental Tryptic Fragment
Use this to affinity purify antibodies

Figure 6

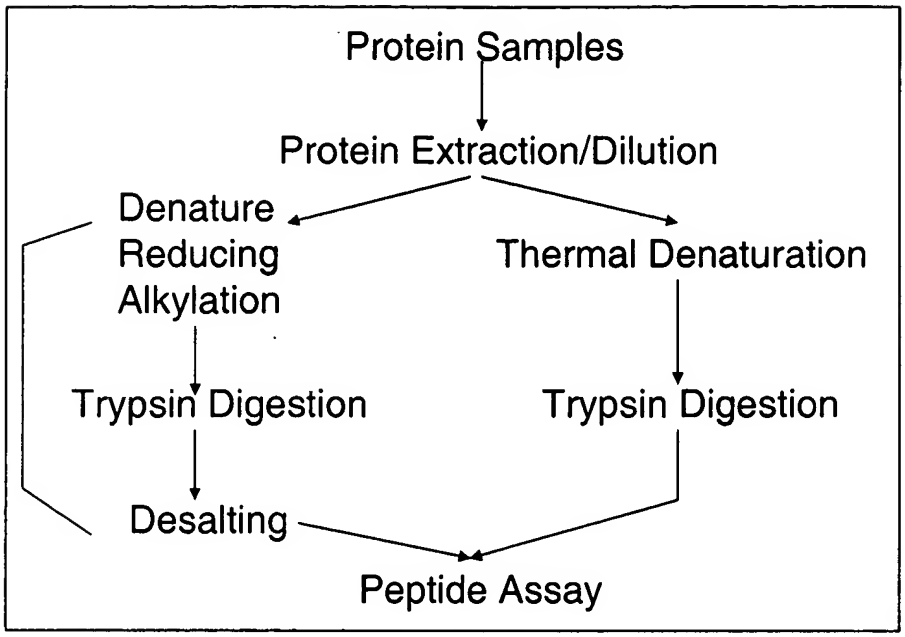

Protein Samples
Protein Extraction/Dilution
Denature
Reducing
Alkylation
Thermal Denaturation
Trypsin Digestion
Trypsin Digestion
Desalting
Peptide Assay

Figure 7

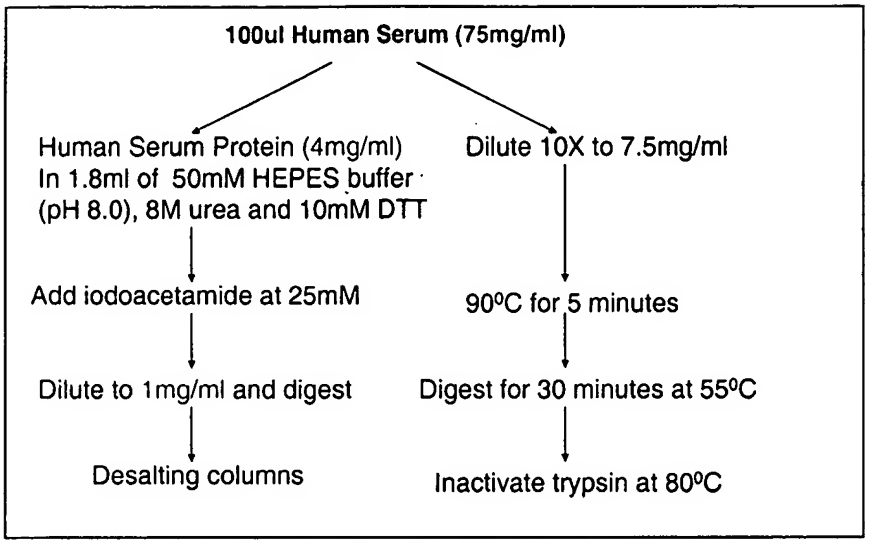

100ul Human Serum (75mg/ml)
Human Serum Protein (4mg/ml)
In 1.8ml of 50mM HEPES buffer
(pH 8.0), 8M urea and 10mM DTT
Dilute 10X to 7.5mg/ml
Add iodoacetamide at 25mM
90°C for 5 minutes
Dilute to 1mg/ml and digest
Digest for 30 minutes at 55°C
Desalting columns
Inactivate trypsin at 80°C

Figure 8

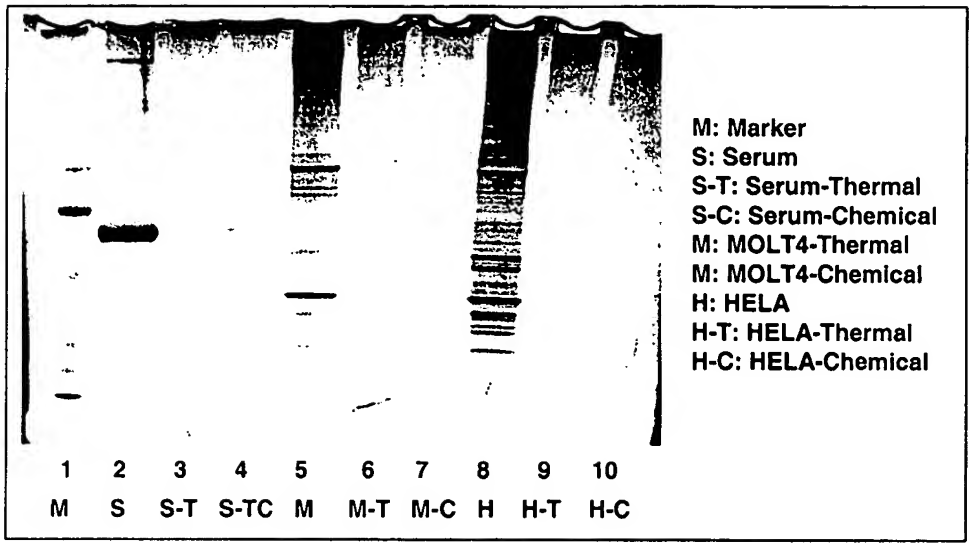

M: Marker
S: Serum
S-T: Serum-Thermal
S-C: Serum-Chemical
M: MOLT4-Thermal
M: MOLT4-Chemical
H: HELA
H-T: HELA-Thermal
H-C: HELA-Chemical
1 2 3 4 5 6 7 8 9 10
M S S-T S-TC M M-T M-C H H-T H-C

Figure 9

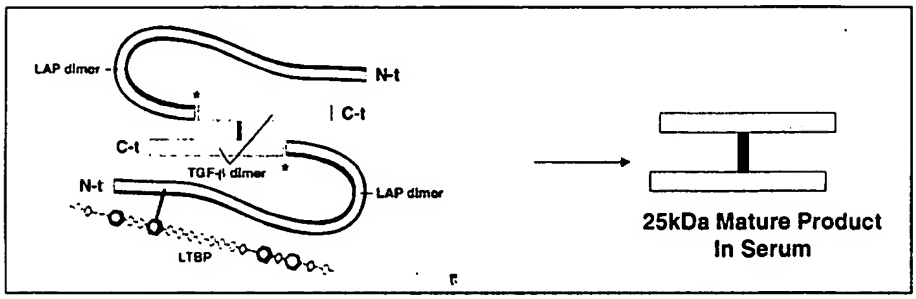

LAP dimer
N-t
C-t
C-t
TGF-β dimer
N-t
LAP dimer
LTBP
25kDa Mature Product
In Serum

Figure 10

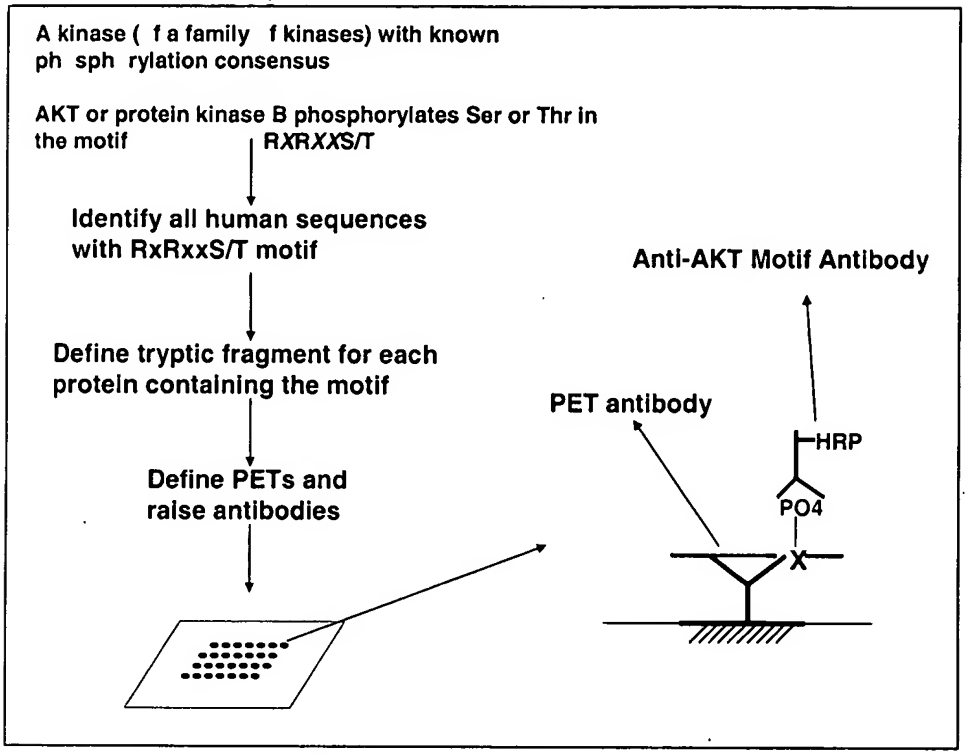

A kinase (f a family f kinases) with known ph sph rylation consensus
AKT or protein kinase B phosphorylates Ser or Thr in the motif RXRXXS/T
Identify all human sequences with RxRxxS/T motif
Define tryptic fragment for each protein containing the motif
Define PETs and raise antibodies
Anti-AKT Motif Antibody
PET antibody
HRP
PO4
X

Figure 11

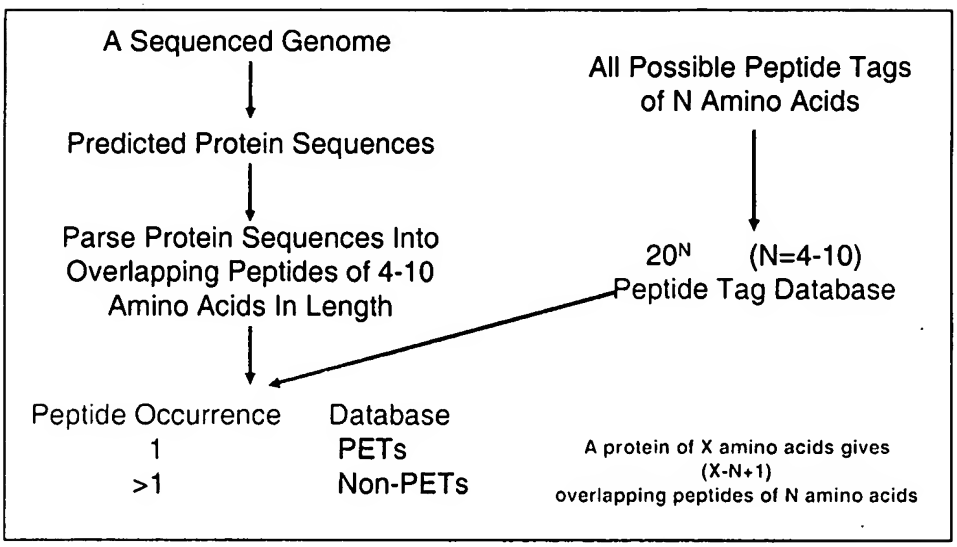

A Sequenced Genome
Predicted Protein Sequences
Parse Protein Sequences Into Overlapping Peptides of 4-10 Amino Acids In Length
All Possible Peptide Tags of N Amino Acids
20^N (N=4-10)
Peptide Tag Database
Peptide Occurrence
1
>1
Database
PETs
Non-PETs
A protein of X amino acids gives (X-N+1) overlapping peptides of N amino acids

Figure 12

Tag Length (amino acids)	All Possible Tags	Total PETs	Total Non-PETs (non-redundant)
4	160,000	745	158,862
5	3.2×10^6	560,309	1,684,684
6	6.4×10^7	4,609,172	2,350,532
7	1.28×10^9	6,652,224	1,848,908
8	2.56×10^{10}	7,018,340	1,744,029
9	5.12×10^{11}	7,138,933	1,714,971
10	1.02×10^{13}	7,216,090	1,695,512

29,076 human protein sequences analyzed
~12M overlapping 4-10mr peptides

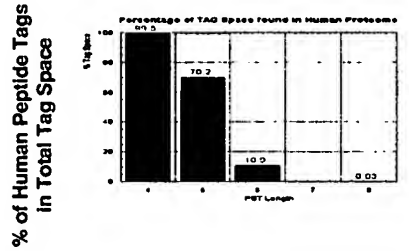


Figure 13

Length of PET (amino acids)	Pr t ins with PETs	Pr teome Coverage
4	684	2.35%
5	23,446	80.64%
6	26,069	89.66%
7	26,184	90.05%
8	26,216	90.16%
9	26,238	90.24%
10	26,250	90.28%

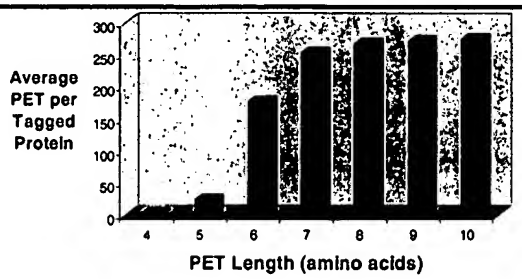


Figure 14

PET Length (Amino Acids)	Average PETs/Tagged Protein No Cleavage	Trypsin Cleavage
5	24	16
6	177	98
8	268	129

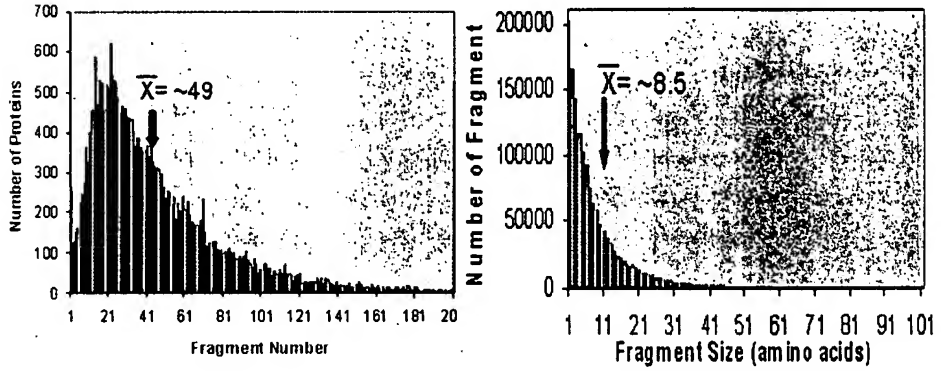


Figure 15

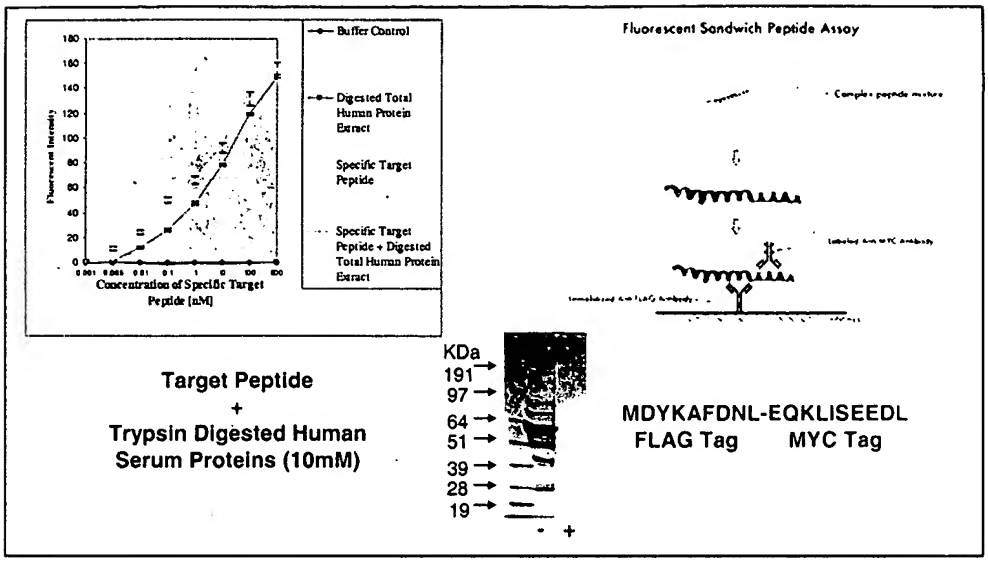

Buffer Control
Digested Total Human Protein Extract
Specific Target Peptide
Specific Target Peptide + Digested Total Human Protein Extract
Concentration of Specific Target Peptide [nM]
Fluorescent Sandwich Peptide Assay
Target Peptide
+
Trypsin Digested Human Serum Proteins (10mM)
KDa
191
97
64
51
39
28
19
MDYKAFDNL-EQKLISEEDL
FLAG Tag
MYC Tag

Figure 16

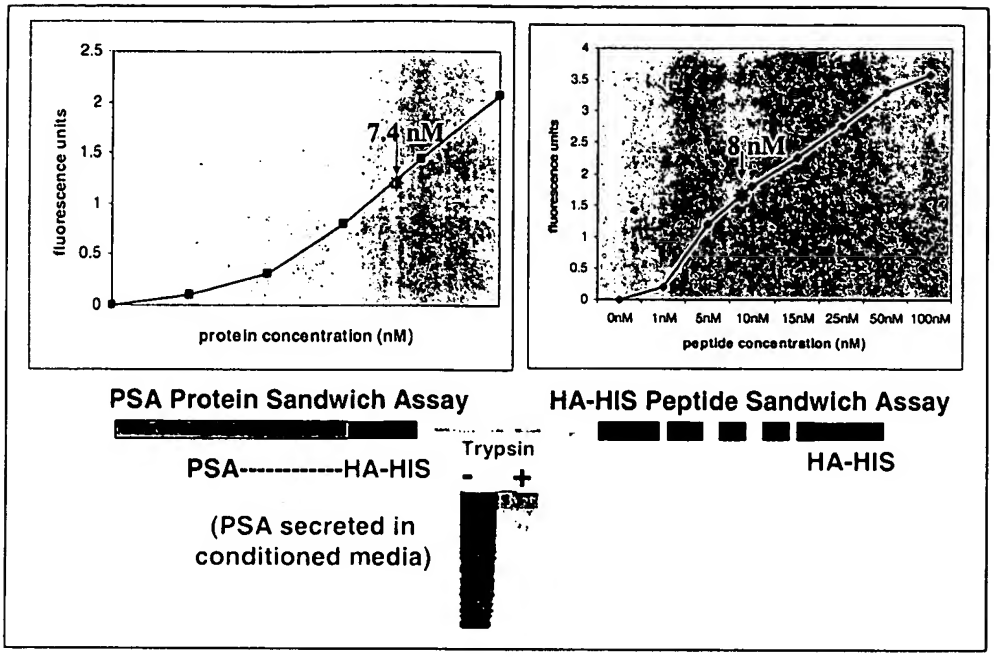

2.5
2
1.5
1
0.5
0
fluorescence units
7.4 nM
protein concentration (nM)
4
3.5
3
2.5
2
1.5
1
0.5
0
fluorescence units
8 nM
0nM 1nM 5nM 10nM 15nM 25nM 50nM 100nM
peptide concentration (nM)
PSA Protein Sandwich Assay
HA-HIS Peptide Sandwich Assay
PSA----------HA-HIS
Trypsin
- +
(PSA secreted in
conditioned media)
HA-HIS

Figure 17

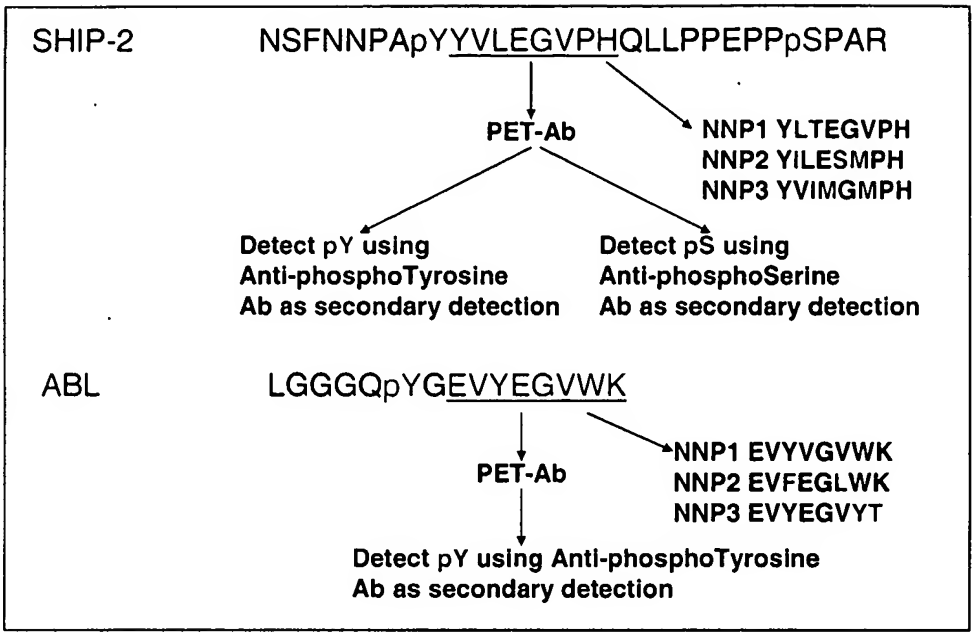
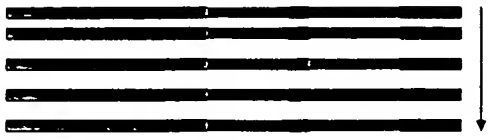
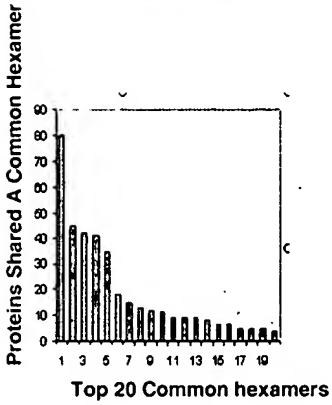

SHIP-2
NSFNNPApYYVLEGVPHQLLPPEPPpSPAR
PET-Ab
NNP1 YLTEGVPH
NNP2 YILESMPH
NNP3 YVIMGMPH
Detect pY using
Anti-phosphoTyrosine
Ab as secondary detection
Detect pS using
Anti-phosphoSerine
Ab as secondary detection
ABL
LGGGQpYGEVYEGVWK
PET-Ab
NNP1 EVYVGVWK
NNP2 EVFEGLWK
NNP3 EVYEGVYT
Detect pY using Anti-phosphoTyrosine
Ab as secondary detection

Figure 18





Size of the Tag	Number To Represent All Kinases
5 aa	88
6 aa	122

Top 20 common hexamers are shared by 72% of 520 kinases

Figure 19

Protein	Parental Tryptic Peptide	Also Detect
BRAF	WSGSHQFEQLSGSILWMAPEVIR*	
DLK	MSFAGTVAWMAPEVIR	
GCK	SFIGTPYWMAPEVAAVER	
HH498	WMAPEVFTQCTR	
HPK1	LSFIGTPYWMAPEVAAVALK	
LOK	DSFIGTPYWMAPEVVMCETMK	KSH1,2,HPK1, SLK
LZK	MSFAGTVAWMAPEVIR	
MAP3K	SMHGTPYWMAPEVINESGYGR	
MST1	NTVIGTPFWMAPEVIQEIGYNCVR	MST2 .
MST4	NTFVGTPFWMAPEVIQQSAYDSK	
MYO3A	NTSVGTPFWMAPEVIACEQQLDTTYDAR	MY03B
MYO3B	NTSVGTPFWMAPEVIACEQQYDSSYDAR	MY03A
ZC1/HGK	NTFIGTPYWMAPEVIACDENPDATYDYR	ZC2,ZC3
OSR1	TFVGTPCWMAPEVMEQVR	
PAK1	STMVGTPYWMAPEVVTR	PAK2,3
PAK5	SLVGTPYWMAPEVISR	PAK6
RAF1	WSGSQQVEQPTGSVLWMAPEVIR	
STLK3	TFVGTPCWMAPEVMEQVR	
TAO1	ASMASPANSFVGTPYWMAPEVILAMDEGQYDGK	TAO3
TAO2	ASIMAPANSFVGTPYWMAPEVILAMDEGQYDGK	
TESK1	EPLAVVGSPYWMAPEVLR	
ZAK	TTHMSLVGTFPWMAPEVIQSLR	

BLUE = PET
RED = Commone Epilope

Figure 20

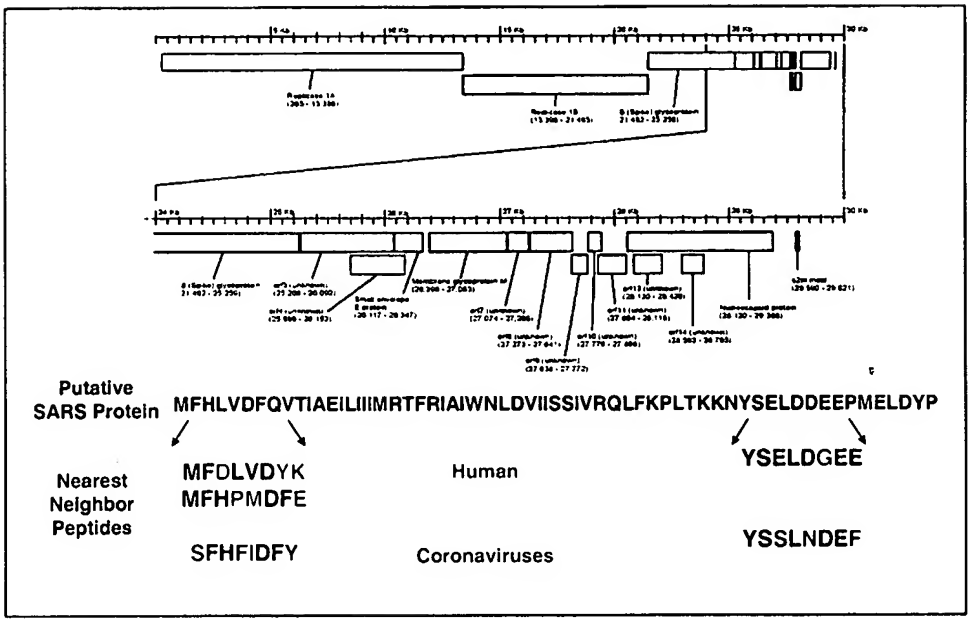

Putative SARS Protein
MFHLVDFQVTIAEILIIMRTFRIAIWNLDVIISSIVRQLFKPLTKKNYSELDDEEPMELDYP
Nearest Neighbor Peptides
MFDLVDYK
MFHPMDFE
SFHFIDFY
Human
Coronaviruses
YSELDGEE
YSSLNDEF

Figure 21

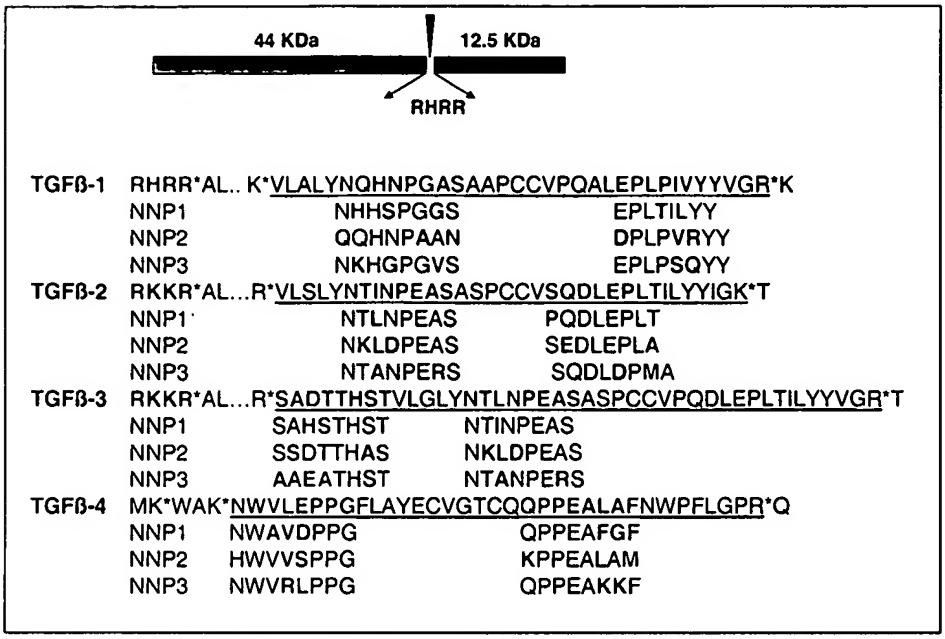

44 KDa
12.5 KDa
RHRR
TGFß-1 RHRR*AL.. K*VLALYNQHNPGASAAPCCVPQALEPLPIVYYVGR*K
NNP1 NHHSPGGS EPLTILYY
NNP2 QQHNPAAN DPLPVRYY
NNP3 NKHGPGVS EPLPSQYY
TGFß-2 RKKR*AL...R*VLSLYNTINPEASASPCCVSQDLEPLTILYYIGK*T
NNP1 NTLNPEAS PQDLEPLT
NNP2 NKLDPEAS SEDLEPLA
NNP3 NTANPERS SQDLDPMA
TGFß-3 RKKR*AL...R*SADTTHSTVLGLYNTLNPEASASPCCVPQDLEPLTILYYVGR*T
NNP1 SAHSTHST NTINPEAS
NNP2 SSDTTHAS NKLDPEAS
NNP3 AAEATHST NTANPERS
TGFß-4 MK*WAK*NWVLEPPGFLAYECVGTCQQPPEALAFNWPFLGPR*Q
NNP1 NWAVDPPG QPPEAFGF
NNP2 HWVVSPPG KPPEALAM
NNP3 NWVRLPPG QPPEAKKF

Figure 22

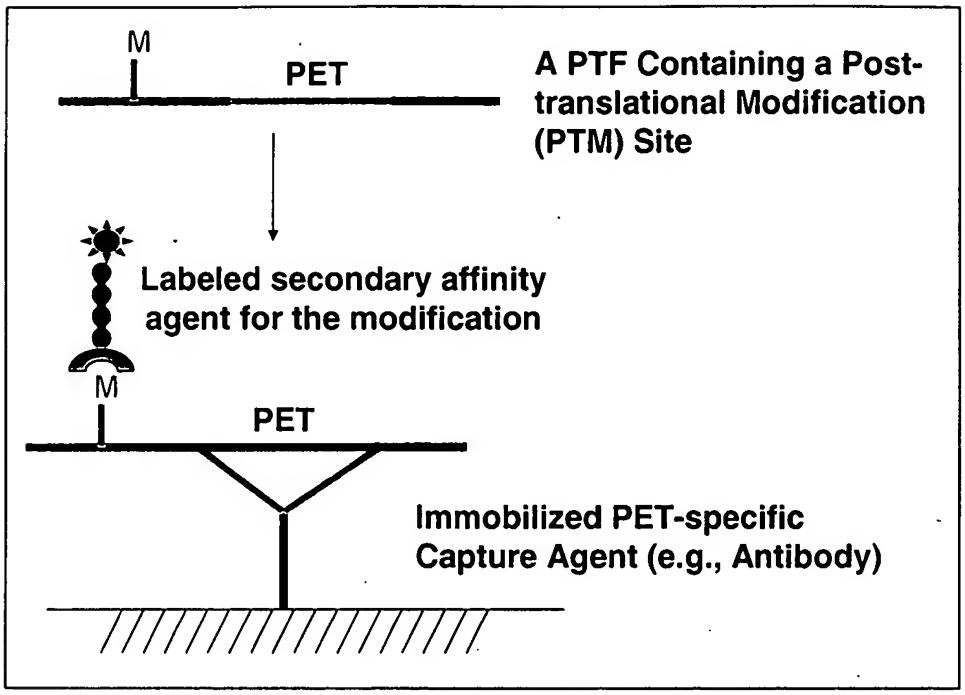

M
PET
A PTF Containing a Post-translational Modification (PTM) Site
Labeled secondary affinity agent for the modification
M
PET
Immobilized PET-specific Capture Agent (e.g., Antibody)

Figure 23

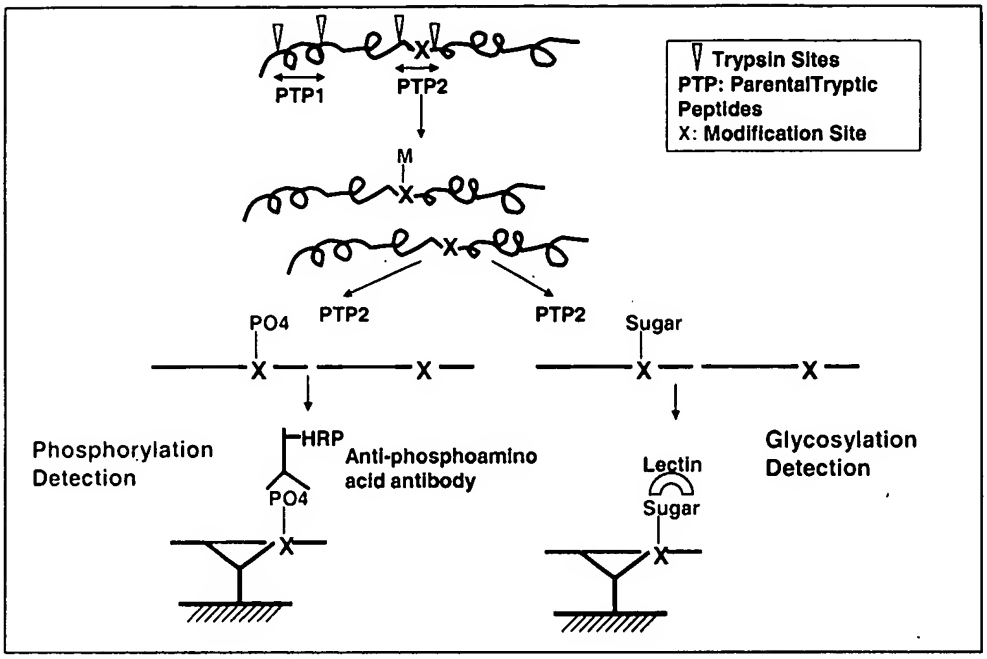
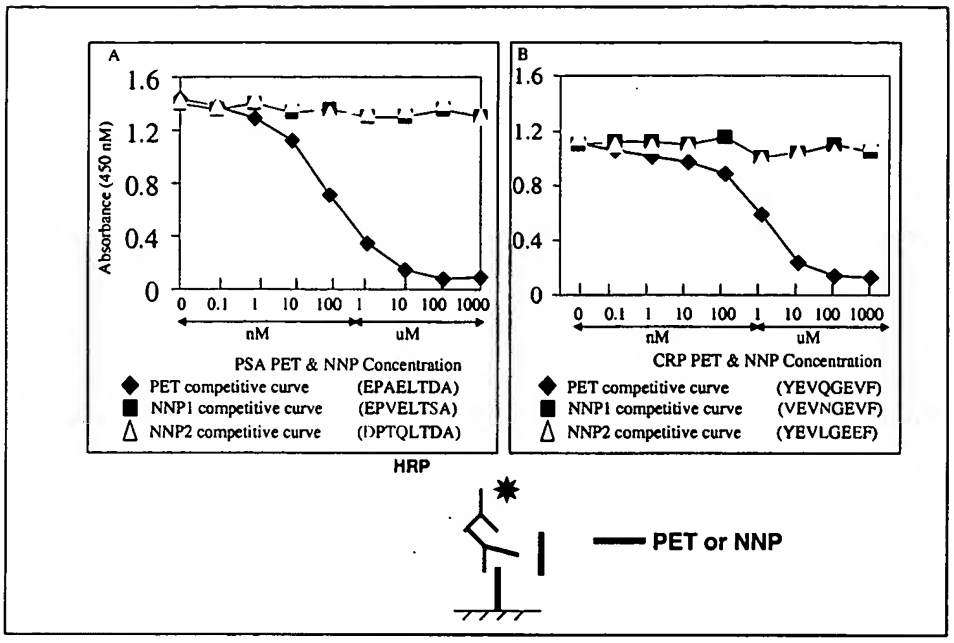

Trypsin Sites
PTP: ParentalTryptic Peptides
X: Modification Site
PTP1
PTP2
M
PO4
PTP2
PTP2
Sugar
Phosphorylation Detection
HRP
Anti-phosphoamino acid antibody
PO4
Lectin
Sugar
Glycosylation Detection

Figure 24


A
1.6
1.2
0.8
0.4
0
Absorbance (450 nM)
0 0.1 1 10 100 1 10 100 1000
nM
uM
PSA PET & NNP Concentration
PET competitive curve (EPAELTDA)
NNP1 competitive curve (EPVELTSA)
NNP2 competitive curve (DPTQLTDA)
B
1.6
1.2
0.8
0.4
0
0 0.1 1 10 100 1 10 100 1000
nM
uM
CRP PET & NNP Concentration
PET competitive curve (YEVQGEVF)
NNP1 competitive curve (VEVNGEVF)
NNP2 competitive curve (YEVLGEEF)
HRP
PET or NNP